Revised Robust Summaries for

Sunset Yellow

CAS No. 2783-94-0

Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:
The International Association of Color Manufacturers/HPV Committee
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List of Member Companies

Colorcon

Noveon, Inc.

Sensient Colors, Inc.

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for Sunset Yellow

The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al*., 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:

- Reliability code 1. Reliable without restrictions
- Reliability code 2. Reliable with restrictions
- Reliability code 3. Not reliable
- Reliability code 4. Not assignable

1 CHEMICAL AND PHYSICAL PROPERTIES

1.1 MELTING POINT

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for substance	FD&C Yellow 6; 91.9% purity
Method/guideline	Measured
GLP	Yes
Year	1981
Remarks for Test Conditions	
Melting Point	
Decomposition	390 °C
Sublimation	
Remarks for Results	Decomposes without melting; decomposition begins at 390 °C

Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for substance	FD&C Yellow 6
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Melting Point	350 °C
Decomposition	
Sublimation	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

1.2 BOILING POINT

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6

Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Boiling Point	837 °C
Pressure	
Pressure Unit	
Decomposition	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

1.3 VAPOR PRESSURE

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for substance	FD&C Yellow 6
Method/guideline	Calculated/Mean of Antoine & Grain
GLP	No
Year	
Remarks for Test Conditions	
Vapor Pressure	1.43 X 10-22 mm Hg
Temperature	25 °C
Decomposition	

Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

1.4 N-OCTANOL/WATER PARTITION COEFFICIENTS

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for substance	FD&C Yellow No. 6
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Log Pow	-1.18
Temperature	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	KOWWIN EPI Suite (2000) US Environmental Protection Agency.

1.5 WATER SOLUBILITY

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Purity not given
Method/guideline	Experimental
GLP	Ambiguous
Year	1991
Remarks for Test Conditions	Not given
Value (mg/L) at temperature	190,000 mg/L at 2 °C, 190,000 mg/L at 25 °C, and 200,000 mg/L at 60 °C
Description of Solubility	Not given
pH value and concentration at temp	
pKa value at 25 Celsius	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Marmion D.M. (1991) Handbook of U.S. Colorants: Foods, Drugs, and Cosmetics and Medical Devices. 3rd Ed. New York, John Wiley & Sons, Inc.

2 ENVIRONMENTAL FATE AND PATHWAYS

2.1 PHOTODEGRADATION

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for structurally related sulfonic acid, 2-naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt (FD&C Red 40)
Method/guideline	Not given
Test Type	Experimental
GLP	Ambiguous
Year	1991
Light Source	15-watt General Electric germicidal lamps
Light Spectrum (nm)	Ultraviolet
Relative Intensity	
Spectrum of Substance	
Remarks for Test Conditions	The concentration of the dye solution was measured before and after the photolysis using the Hewlett-Packard 8452A diode-array UV/Visible Spectrophotometer. Red 40 was prepared in an initial concentration of 5 mg/l. In the first part of the study, photolysis experiments were conducted using two 15-W (30 Watts total) General Electric germicidal lamps as the ultraviolet light source. The distance between the light source and the reaction vessels was approximately 2.5 cm. Both direct photolysis and indirect photolysis experiments were conducted. The indirect photolysis experiment used acetone as the sensitizer for indirect photodegradation.
Concentration of Substance	5 mg/L
Temperature	
Direct photolysis	7% degradation after 50 minutes
Halflife t1/2	
Degradation % after	
Quantum yield	
Indirect photolysis	99% degradation after 20 minutes
Sensitizer	Acetone

Concentration of sensitizer	5 mg/L
Rate constant	
Degradation %after	
Breakdown products	
Remarks field for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Pasin B. and Rickabaugh J. (1991) Destruction of Azo Dyes by Sensitized Photolysis. Hazard. Ind. Wastes, 359-367.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6
Method/guideline	Calculation
Test Type	AOPWIN
GLP	
Year	
Light Source	
Light Spectrum (nm)	
Relative Intensity	
Spectrum of Substance	
Remarks for Test Conditions	
Concentration of Substance	
Temperature	
Direct photolysis	
Halflife t1/2	31.9 hours
Degradation % after	
Quantum yield	
Indirect photolysis	

Sensitizer	
Concentration of sensitizer	
Rate constant	
Degradation %after	
Breakdown products	
Remarks field for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	AOPWIN EPI Suite (2000) US Environmental Protection Agency.

2.2 BIODEGRADATION

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for structurally related sulfonic acid C.I. Acid Red No. 9(benzenesulfonic acid 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methyl, barium salt (CAS No-5160-02-1); Assay, 90%
Method	OECD 301C
Test Type	
GLP	Ambiguous
Year	1992
Contact time (units)	28 days
Innoculum	Activated sludge
Remarks for Test Conditions	Standard OECD 301C guideline study
Results	Not biodegradable

Classification

Remarks fields for results	In Zahn-Wellens test, after 21 days, 33% was loss with 10% absorbed on the sludge.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	OECD SIDS (1999) 9th SIAM for D&C Red No. 9

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for structurally related substance C.I. Acid Red No. 14
Method	Not given
Test Type	
GLP	Ambiguous
Year	1993
Contact time (units)	24 hour
Innoculum	Activated sludge
Remarks for Test Conditions	Screened raw wastewater was used as the influent in three pilot scale activated sludge biological treatment systems. Each water soluble dye was tested at doses of 1 mg/L for low spike systems and 5 mg/L for high spike systems of influent flow. Before the data collection, dye analytical recovery studies were conducted by dosing the purified dye compound into organic free water, influent wastewater, and mixed liquor. These studies were run in duplicate and each recovery study was repeated at least once to ensure that the dye compound could be extracted. Purified dye standards were analytically prepared from the commercial dye product by repeated recrystallization. The INF, primary effluent (PE), and ASE were filtered and the filtrate was passed through a column packed with resin. The filter paper and resin were soaked in an ammonia acetonitrile solution and then Soxhlet extracted with ammonia-acetonitrile. The extract was concentrated and brought up to 50 mL volume with a methanol/dimethylformamide solution. The mixed liquor samples were separated into two components, the filtrate or soluble fraction (SOL) and the residue (RES) fraction. The SOL fraction was processed similar to these samples but he resin adsorption step was omitted. All extracted samples were analyzed by HPLC with and ultraviolet-visible detector. Total suspended solids analyses were also performed on the INF, PE, ML, and ASE samples.

	All systems were operated for at least three times the solids retention time to ensure acclimation prior to initiation of data collection. All samples were 24 hr. composites made up of 6 grab samples collected every 4 hr. and stored at 4 degrees Celsius.
Degradation % after time	
Results	Percent recovery as measured: Organic Free Water: 101% at 1 mg/L and 90% at 5 mg/L; Wastewater: 98% at 1mg/L and 97% at 5 mg/L; Mixed Liquor: 88% at 1mg/L and 92% at 5 mg/L Mass Balance Data Summary: Low spike: 116% recovered, 1% adsorbed; High spike: 148% recovered, less than 1% adsorbed.
Kinetic	
Time required for 10% degradation **10 day window criteria**	
Total degradation	
Classification	
Breakdown products (transient or stable?)	
Remarks fields for results	Since the majority of the test substance was recovered, the authors assumed that this compound was not biodegraded. The authors based this assumption on preliminary data indicating little or no problems in recovering the compounds from the sample matrix. Additionally, the results also indicate that the material was not adsorbed. The authors attributed the high sulfonic acid substitution on the test substance as the reason why the material was not removed by the microbial cells or cell byproducts and subject to aerobic biodegradation.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Shaul G.M., Holdsworth T.J., Dempsey C.R., and Dostal K.A. (1990) Fate of water soluble azo dyes in the activated sludge process. Chemosphere 22, p107-119.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD & C Yellow 6
Method	BIOWIN
Test Type	Calculated

GLP	
Year	
Contact time (units)	
Innoculum	
Remarks for Test Conditions	
Degradation % after time	
Results	
Kinetic	
Time required for 10% degradation **10 day window criteria**	
Total degradation	
Classification	Not readily biodegradable
Breakdown products (transient or stable?) **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	BIOWIN EPI Suite (2000) US Environmental Protection Agency.

2.3 FUGACITY

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model

Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Air
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration	0.00219%
Remarks	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Soil
absorption coefficient	

Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration Remarks	50.1%
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Water
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration Remarks	49.8%
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Sediment
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration	0.0918%
Remarks	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

3 ECOTOXICITY

3.1 ACUTE TOXICITY TO FISH

Substance Name	Sunset Yellow
CAS No.	2783-94-0
Remarks for Substance	Data are for structurally related azo dye, benzenesulfonic acid 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methyl, barium salt (CAS No-5160-02-1); Assay, 90%
Test Type	Experimental (semi-static) Method 84/449/EEC
GLP	Yes
Year	1982
Species/Strain/Supplier	Fish (Oryzias latipes) (Orange killifish)
Exposure Period	96 hour
Remarks for Test Condition	A group of 10 fish were exposed to 5 nominal concentrations. Two controls, DMSO(0.5 mg/L) and lab water were used
Endpoint value	96-hr LC50 = >420 mg/L
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Hoechst AG (1992). Unveroeffentlichte Untersuchung (82.0250).

Substance Name	Sunset Yellow
CAS No.	2783-94-0
Remarks for Substance	Data are for structurally related azo dye, benzenesulfonic acid 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methyl, barium salt (CAS No-5160-02-1); Assay, 90%
Test Type	Experimental (static) Method 84/449/EEC
GLP	Yes
Year	1982
Species/Strain/Supplier	Fish (Brachydanio rerio)
Exposure Period	96 hour

Remarks for Test Condition	A group of 10 fish were exposed to 5 nominal concentrations. Two controls, DMSO(0.5 mg/L) and lab water were used
Endpoint value	96-hr LC50 = >500 mg/L
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Hoechst AG (1992). Unveroeffentlichte Untersuchung (82.0250).

Substance Name	Sunset Yellow
CAS No.	2783-94-0
Remarks for Substance	Data are for structurally related azo dye, D&C Red No. 7, 2-naphthalenecarboxylic acid, [(4-methyl-2-sulfophenyl)azo], calcium salt acid (CAS No-5281-04-9); Assay, 87%
Test Type	Experimental (flow-through) Japanese Industrial Standard (JIS K 0102-1986)
GLP	Yes
Year	1992
Species/Strain/Supplier	Fish (Oryzias latipes) (Orange killifish)
Exposure Period	96 hour
Remarks for Test Condition	NA
Endpoint value	48-hr LC50 = 50 mg/L
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
Reference	MITI, Japan (1992).

Substance Name	Sunset Yellow
CAS No.	2783-94-0
Remarks for Substance	Data are for structurally related azo dye, D&C Red No. 7, 2-naphthalenecarboxylic acid, [(4-methyl-2-sulfophenyl)azo], calcium salt acid (CAS No-5281-04-9); Assay, 87%
Test Type	Experimental (OECD Guideline 203-semi-static-open system)
GLP	Ambiguous
Year	Not given

Species/Strain/Supplier	Fish (Oryzias latipes) (Orange killifish)
Exposure Period	96 hour
Remarks for Test Condition	A group of 10 fish were exposed to 5 nominal concentrations of 17.1 to180 mg/L. Two controls, DMSO(0.5 mg/L) and lab water were used
Endpoint value	96-hr LC50 = 33 mg/L (95% C.I., 11-98 mg/L)
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
Reference	EA, Japan (1992).

Substance Name	Sunset Yellow
CAS No.	2783-94-0
Remarks for Substance	Data are for structurally related azo dye, benzenesulfonic acid 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methyl, barium salt (CAS No-5160-02-1); Assay, 90%
Test Type	Experimental (semi-static) Method 84/449/EEC
GLP	Yes
Year	1982
Species/Strain/Supplier	Fish (Oryzias latipes) (Orange killifish)
Exposure Period	96 hour
Remarks for Test Condition	A group of 10 fish were exposed to 5 nominal concentrations. Two controls, DMSO(0.5 mg/L) and lab water were used
Endpoint value	96-hr LC50 = >500 mg/L
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Hoechst AG (1992). Unveroeffentlichte Untersuchung (82.0250).

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for sulfonic acid derivative, 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid
Method/guideline	
Test Type	Experimental

GLP	Ambiguous
Year	Not given
Species/Strain/Supplier	Fish
Analytical monitoring	
Exposure period (unit)	48 hour
Remarks for Test Conditions	
Observations on precipitation **Nominal concentrations as mg/L** **Measured concentrations as mg/L** **Unit**	
Endpoint value	LC50 = 200 mg/L
Reference substances (if used) **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt 1992. Schön N. (1991) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 3(3), 183-185. Schön N. (1992) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 4(6), 343-345.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for sulfonic acid derivative,2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, disodium salt
Method/guideline	
Test Type	Experimental
GLP	Ambiguous

Year	Not given
Species/Strain/Supplier	Fish
Analytical monitoring	
Exposure period (unit)	72 hour
Remarks for Test Conditions	
Observations on precipitation **Nominal concentrations as mg/L** **Measured concentrations as mg/L** **Unit**	
Endpoint value	LC50 greater than 1000 mg/L
Reference substances (if used) **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt 1992. Schön N. (1991) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 3(3), 183-185. Schön N. (1992) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 4(6), 343-345.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for sulfonic acid derivative, 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, dipotassium salt
Method/guideline	
Test Type	Experimental
GLP	Ambiguous
Year	Not given

Species/Strain/Supplier	Fish
Analytical monitoring	
Exposure period (unit)	96 hour
Remarks for Test Conditions	
Observations on precipitation **Nominal concentrations as mg/L** **Measured concentrations as mg/L** **Unit**	
Endpoint value	LC50 greater than 10000 mg/L
Reference substances (if used) **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt 1992. Schön N. (1991) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 3(3), 183-185. Schön N. (1992) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 4(6), 343-345.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Species/Strain/Supplier	Fish

Analytical monitoring	
Exposure period (unit)	96 hour
Remarks for Test Conditions	Input parameters: Molecular weight, Water solubility, 190,000 mg/L at 25 °C; melting point 390 °C
Observations on precipitation	
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	LC50 = 6044 mg/L
Reference substances (if used)	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

3.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Substance Name	Sunset Yellow
CAS No.	2783-94-0
Remarks for Substance	Data are for structurally related azo dye, benzenesulfonic acid 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methyl, barium salt (CAS No-5160-02-1); Assay, 90%
Test Type	Experimental OECD 202
GLP	Yes
Year	1992
Species/Strain/Supplier	Dapnid (Daphnia magna)

Exposure Period	48 hour
Remarks for Test Condition	Saturated solution of test material was used
Endpoint value	48-hr EC50 = >2 mg/L
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Hoechst AG (1993). Unveroeffentlichte Untersuchung (93.0358).

Substance Name	Sunset Yellow
CAS No.	2783-94-0
Remarks for Substance	Data are for structurally related azo dye, D&C Red No. 7, 2-naphthalenecarboxylic acid, [(4-methyl-2-sulfophenyl)azo], calcium salt acid (CAS No-5281-04-9); Assay, 87%
Test Type	Experimental (static) OECD 202 Guideline Study
GLP	No
Year	1984
Species/Strain/Supplier	Daphnid (Daphnia magna)
Exposure Period	24 hour
Remarks for Test Condition	20 daphnids(4 replicates, 5 organisms per plate) were exposed to 5 nominal concentrations of 90-940 mg/L. Control was DMSO;DCO40=9:1 (100 mg/L) and lab water.
Endpoint value	24-hr EC50 = 280 mg/L (95% C.I.=150-490 mg/L)
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
Reference	EA, Japan (1992).
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for sulfonic acid derivative, 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid
Method/guideline	
Test Type	Experimental
GLP	
Year	

Analytical procedures	
Species/Strain	*Daphnia magna*
Test details	24 hour
Remarks for Test Conditions	
Nominal concentrations as mg/L **Measured concentrations as mg/L** **Unit**	
EC50, EL50, LC0, at 24,48 hours **Biological observations**	EC50 = 100 mg/L
Control response satisfactory? **Appropriate statistical evaluations?** **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt 1992. Schön N. (1991) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 3(3), 183-185. Schön N. (1992) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 4(6), 343-345.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	

Analytical procedures	
Species/Strain	*Daphnia magna*
Test details	48 hours
Remarks for Test Conditions	Input parameters: Water solubility, 190,000 mg/L at 25 °C; Molecular weight 452.37; Melting point 390 °C
Nominal concentrations as mg/L **Measured concentrations as mg/L** **Unit**	
EC50, EL50, LC0, at 24,48 hours **Biological observations**	EC50 = 486.5 mg/L
Control response satisfactory? **Appropriate statistical evaluations?** **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

3.3 ACUTE TOXICITY TO AQUATIC PLANTS

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	The test substance was an unidentified sulfonic acid substituted azo dye.
Method/guideline	
Test Type	Experimental
GLP	Ambiguous

Year	1996
Species/Strain/Supplier	Green algae, *Selenatrum capricornutum*
Endpoint basis	
Exposure period (duration)	96 hour
Analytical monitoring	
Remarks for Test Conditions	Algal chronic toxicity test were performed according the method of EPA, 1988. Three replicates were performed for each dye at a nominal concentration of 1 mg/l for the active colorant. One ml of dye stock solution was added to 50 mg/l of algal assay medium in 125 ml Erlenmeyer flasks. S. capricornutum in continuous culture provided the initial innoculum (10,000 algal cells/ml). The cells were incubated in the solution for 96 hours. The diluent and negative control were algal assay medium. AAM was prepared by adding 1 ml from each of five stock solutions to 900 ml of deionized water. After spiking, the total volume was brought to 1 liter with deionized water. Population growth was used to establish potential toxicity. If the dye inhibited algal growth by more than 50% of that of the negative controls, a definitive test using several dilutions of the dye was performed to allow for determination of an EC50 concentration.
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	Average yield: 36.6% with 95% C.I. (34.9-38.4).
NOEC, LOEC or NOEL, LOEL	
Biological observations	26.4% stimulation of population growth compared to control.
Control response satisfactory?	Yes
Appropriate statistical evaluations?	Yes, Dunnett's test
Remarks fields for results	Not statistically significant.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Greene J. C. and Baughman G.L. (1996) Effects of 46 dyes on population-growth of fresh-water green-alga selenastrum-capricornutum. Textile Chemist And Colorist, 28, 23-30. Green J.D. et al. (1988) Protocols for short term toxicity screening of hazardous w

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Species/Strain/Supplier	Green algae
Endpoint basis	
Exposure period (duration)	96 hour
Analytical monitoring	
Remarks for Test Conditions	Input parameters: Water solubility - 190,000 mg/L at 25 °C; Molecular weight 452.37; Melting point 390 °C
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	EC50 = 146,000 mg/L
NOEC, LOEC or NOEL, LOEL	
Biological observations	
Control response satisfactory?	
Appropriate statistical evaluations?	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) US Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

4 HUMAN HEALTH TOXICITY

4.1 ACUTE TOXICITY

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Not given
Method/guideline	Not given
Test Type	Acute Toxicity LD50
GLP	No
Year	1964
Species/Strain	Rats/Wistar
Sex	Male
# of animals per sex per dose	6
Vehicle	Water
Route of administration	Oral-Gavage
Remarks for test conditions	Wistar adult male rats were administered 2000 mg/kg bw *via* stomach tube.
Value LD50 or LC50 with confidence limits	Greater than 2000 mg/kg bw
Number of deaths at each dose level	0 deaths
Remarks for results	
Conclusion remarks	The oral LD50 for sunset yellow is greater than 2000 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Lu F. and Lavalle C. (1964) The acute toxicity of some synthetic colours used in drugs and foods. Canadian Pharmaceutical Journal 9.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Greater than 85% purity
Method/guideline	LD50 calculated by Weil (1952)

Test Type	Acute Toxicity LD50
GLP	No
Year	1967
Species/Strain	Rats/Carworth Farm E strain
Sex	Male and Female
# of animals per sex per dose	5
Vehicle	Water
Route of administration	Oral
Remarks for test conditions	Groups of five male and female rats each (body weights: males 200-250 g; females 150-200 g) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors.
Value LD50 or LC50 with confidence limits	Greater than 10,000 mg/kg
Number of deaths at each dose level	No deaths at up to 10,000 mg/kg bw.
Remarks for results	Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Gaunt I.F., Farmer M., Grasso P., and Gangolli .D. (1967) Acute (Rat and Mouse) and Short-term (Rat) Toxicity Studies on Sunset Yellow FCF. Fd Cosmet Toxicol 5, pp. 747-754.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Greater than 85% purity
Method/guideline	LD50 calculated by Weil (1952)
Test Type	Acute Toxicity LD50
GLP	No
Year	1967
Species/Strain	Mice/ICI Alderley Park strain
Sex	Male and Female

# of animals per sex per dose	5
Vehicle	Water
Route of administration	Oral
Remarks for test conditions	Groups of five male and female mice each (body weights: 20-25 kg) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors.
Value LD50 or LC50 with confidence limits	Greater than 6000 mg/kg bw
Number of deaths at each dose level	No deaths at up to 6000 mg/kg bw
Remarks for results	Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Gaunt I.F., Farmer M., Grasso P., and Gangolli .D. (1967) Acute (Rat and Mouse) and Short-term (Rat) Toxicity Studies on Sunset Yellow FCF. Fd Cosmet Toxicol 5, pp. 747-754.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Greater than 85% purity
Method/guideline	LD50 calculated by Weil (1952)
Test Type	Acute Toxicity LD50
GLP	No
Year	1967
Species/Strain	Rats/Carworth Farm E strain
Sex	Male and Female
# of animals per sex per dose	5
Vehicle	Water
Route of administration	Intraperitoneal
Remarks for test conditions	Groups of five male and female rats each (body weights: males 200-250 g; females 150-200 g) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died

	and some survivors.
Value LD50 or LC50 with confidence limits	3800 mg/kg bw (2900-4600 mg/kg bw)
Number of deaths at each dose level	Not given
Remarks for results	Slight diarrhea reported for 24 hours following treatment. Skin, feces and urine were colored orange. Deaths were preceded by comas, and in some animals convulsions. No macroscopic changes reported upon necropsy.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Gaunt I.F., Farmer M., Grasso P., and Gangolli .D. (1967) Acute (Rat and Mouse) and Short-term (Rat) Toxicity Studies on Sunset Yellow FCF. Fd Cosmet Toxicol 5, pp. 747-754.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Greater than 85% purity
Method/guideline	LD50 calculated by Weil (1952)
Test Type	Acute Toxicity LD50
GLP	No
Year	1967
Species/Strain	Mice/ICI Alderley Park strain
Sex	Male and Female
# of animals per sex per dose	5
Vehicle	Water
Route of administration	Intraperitoneal
Remarks for test conditions	Groups of five male and female mice each (body weights: 20-25 kg) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors.
Value LD50 or LC50 with confidence limits	5500 (95% C.I.: 4600-6700) mg/kg bw (Males) 4600 (95% C.I.: 3900-5300) (Females)
Number of deaths at each dose level	Not given
Remarks for results	Slight diarrhea reported for 24 hours following treatment. Skin, feces and urine were colored orange. Deaths were preceded by comas, and in some animals convulsions. No macroscopic

	changes reported upon necropsy.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Gaunt I.F., Farmer M., Grasso P., and Gangolli .D. (1967) Acute (Rat and Mouse) and Short-term (Rat) Toxicity Studies on Sunset Yellow FCF. Fd Cosmet Toxicol 5, pp. 747-754.

4.2 GENETIC TOXICITY

4.2.1 In vitro Genotoxicity

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6; Purity not given
Method/guideline	Ames plate incorporation and liquid pre-incubation
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1981
Species/Strain	*Salmonella typhimurium* TA1535, TA 1537, TA1538, TA98, TA100
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/concentration levels	.005- 5.0 mg/plate
Statistical Methods	Not given
Remarks for test conditions	Reverse mutation tests were carried out using S. typhimurium strains TA1535, TA 1537, TA1538, TA98, TA100. Plate incorporation tests were conducted according to Ames et al., with the Andrews et al. modifications. Duplicates were performed at each of the six concentrations of the test substance. Mutagenic compounds were assayed using duplicate plates. A substance was considered positive when

	the number of revertants above background was at least twice the value of the historical control mean or twice the value of the current control mean, whichever was greater and a dose response curve could be generated. Positive controls without metabolic activation were sodium azide (TA1535 and TA100), 9-aminoacridine (TA97 and TA1535), and 4-nitro-o-phenylenediamine (TA98). The positive controls were sodium azide, 9-aminoacridine, 2-nitrofluorene, and 2-aminoanthracene.
Result	Negative Positive control Results (-S9/+S9) Cmpd Amt per plate TA1538 TA98 TA100 None 9/24 11/35 100/87 DMSO .1 ml 11/19 21/27 124/99 Sodium azide 0.5 ug 13/20 11/23 1165/96 2-Nitrofluorene 5 ug 728/239 578/171 1586/525 2-Aminoanthracene 2.5 ug 15/882 22/799 90/2593
Cytotoxic concentration	5.0 mg/plate for plate-incorporation, and .5 mg/ml for pre-incubation test
Genotoxic effects	Negative
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	The test substance was negative in the AMES assay for reverse mutation using Salmonella typhimurium TA1535, TA 1537, TA1538, TA98, TA100.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Chung K.T., Fulk G.E., & Andrews A.W. (1981) Mutagenicity testing of some commonly used dyes. Applied and Environmental Microbiology 42, 641-648.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6; Purity not given
Method/guideline	Ames, McCann and Yamasaki (1975)
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1984
Species/Strain	*Salmonella typhimurium* TA1535, TA 1537, TA98, TA100, TA92, TA94

Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/concentration levels	up to 5.0 mg/ml
Statistical Methods	Not given
Remarks for test conditions	Reverse mutation tests were carried out using S. typhimurium strains TA92, TA1535, TA100, TA1537, TA94 and TA98. Cells cultured overnight were pre-incubated with the test substance and the S-9 mix for twenty minutes at 37 degrees Celsius prior to plating. Duplicates were performed at each of the six concentrations of the test substance. The number of revertant colonies were counted following incubation for two days. Negative controls were either untreated plates or solvent. Positive results were determined if the number of colonies found was twice the number in the control. If the test was positive and a dose response relationship was not detected, additional experiments at different doses or induced mutation frequency assays were performed.
Result	Negative
Cytotoxic concentration	5.0 mg/ml was the highest non-cytotoxic dose used in the experiment.
Genotoxic effects	Negative
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	Sunset Yellow was negative in the AMES assa;y for reverse mutation using Salmonella typhimurium TA1535, TA 1537, TA98, TA100, TA92, TA94.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report which meets basic scientific principles.
References	Ishidate, M., Sofuni, T., Yoshikawa, K., Hauashi, M., Nohmi, T., Sawada, M. and Matsuoka. (1984). Primary Mutagenicity Screening of Food Additives Currently Used in Japan. Fd. Chem. Toxic. 22(8) 623-636.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6; Purity not given
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	No
Year	1979

Species/Strain	*Salmonella typhimurium* TA1535, TA 1537, TA98, TA100
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/concentration levels	10-250 mg/plate
Statistical Methods	Not given
Remarks for test conditions	The test substance was dissolved in DMSO. The test was considered positive if 2 fold increase in revertants was observed. Positive controls included 9-aminoacridine; 2-aminoflourine; and N-methyl-N-nitrosoguanidine.
Result	Negative
Cytotoxic concentration	Not given
Genotoxic effects	Negative
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	No evidence of genotoxicity was reported.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Muzzall J.M. and Cook W.I. (1979) Mutagenicity test of dyes used in cosmetics with the Salmonella/mammalian microsome test. Mutations Research 67, 1-8.a
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6; Purity 91.8%
Method/guideline	Sister Chromatid Exchange test was carried out using a Chinese hamster ovary (CHO).
Test Type	Sister Chromatid Exchange
System of Testing	Chinese hamster ovary cells
GLP	Ambiguous
Year	1989
Species/Strain	Chinese hamster ovary cells (CHO)
Metabolic Activation	With and without metabolic activation
Doses/concentration levels	up to 5,000 micrograms/mL
Statistical Methods	Trend test.
Remarks for test conditions	Sister chromatid exchange tests were carried out using the Chinese hamster ovary cells. Cells were exposed to the test

	substance for 25 hr. With metabolic activation, the cells were exposed to the test chemical plus the metabolic activation for 2 hr. For both tests (with and without metabolic activation) 10 micromolar bromodeoxyuridine (BrdUrd) was added 2 hours following initiation of the test. Colcemid was present for the last 2-2.5 hours of the incubation. Without metabolic activation, the total incubation time was 27.5-28 hr and the cells were washed prior to the addition of the Colcemid. The cultures with metabolic activation were washed to remove the test substance and the metabolic activation 2 hours following initial exposure.
Result	In one trial without activation, SCE's were induced at 30 and 25% respectively at 1,667 and 5,000 micrograms/ml. With activation, the test substance did not induce SCE's at concentrations up to 5000 micrograms/mL.
Cytotoxic concentration	Not given
Genotoxic effects	Equivocal.
Appropriate statistical evaluations?	Yes, trend test
Remarks for results	Equivocal without activation. Negative with activation.
Conclusion remarks	The SCE response to FD&C Yellow No. 6 was judged to equivocal without activation and negative with activation.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report which meets basic scientific principles.
References	Ivett J.L., Brown B.M., Rodgers C., Anderson B.E., Resnick M.A., and Zeigler, E. (1989) Chromosomal aberrations and sister chromatid exchange tests in Chinese Hamster Ovary Cells in Vitro. IV. Results with 15 chemicals. Environmental and Molecular Mut
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6; Purity 91.8%
Method/guideline	Chromosomal aberration test was carried out using a Chinese hamster ovary cell line, CHL.
Test Type	Chromosomal aberration test
System of Testing	Chinese hamster ovary cells
GLP	Ambiguous
Year	1989
Species/Strain	Chinese hamster ovary cells (CHO)
Metabolic Activation	With and without metabolic activation
Doses/concentration levels	up to 5,000 micrograms/L

Statistical Methods	
Remarks for test conditions	Chromosomal aberration tests were carried out using the Chinese hamster ovary cells. Cells were exposed to the test substance for 8 hr. With metabolic activation, the cells were exposed to the test chemical plus the metabolic activation for 2 hr, washed, incubated for 8 hr., and then treated with Colcemid for 2-2.5 hr. The cells were prepared for viewing on slides.
Result	Negative with and without metabolic activation.
Cytotoxic concentration	Not given
Genotoxic effects	Negative
Appropriate statistical evaluations?	Yes, trend test
Remarks for results	Negative
Conclusion remarks	Sunset Yellow tested negative in the chromosomal aberration test using Chinese hamster ovary cells.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report which meets basic scientific principles.
References	Ivett J.L., Brown B.M., Rodgers C., Anderson B.E., Resnick M.A., and Zeigler, E. (1989) Chromosomal aberrations and sister chromatid exchange tests in Chinese Hamster Ovary Cells in Vitro. IV. Results with 15 chemicals. Environmental and Molecular Mut
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6; Purity not given
Method/guideline	Chromosomal aberration test was carried out using a Chinese hamster fibroblast cell line, CHL. The cells were exposed to 3 different doses for 24 and 48 hours. No metabolic activation system was applied.
Test Type	Chromosomal aberration test
System of Testing	Chinese hamster fibroblast cell line CHL.
GLP	Ambiguous
Year	1984
Species/Strain	Chinese hamster fibroblast cell line CHL.
Metabolic Activation	None
Doses/concentration levels	up to 6.0 mg/ml
Statistical Methods	

Remarks for test conditions	Chromosomal aberration tests were carried out using the Chinese hamster fibroblast line. Cells were exposed to the test substance at three different doses for 24 and 48 hr. No metabolic activation was employed. The maximum dose used for each test substance was found in a preliminary test to determine the dose required for 50% cell-growth inhibition. Colcemid at a final concentration of 0.2 ug/ml was added to the culture two hours prior to cell harvesting. The cells were prepared for viewing on slides. One hundred visible metaphases were observed under the microscope and the incidence of polyploid cells and structural chromosomal aberrations (including choromosome and chromatid gaps, breaks, exchanges, ring formations, fragmentations and others were recorded. Negative controls included untreated cells and solvent treated cells. The incidence of aberrations in the negative controls was generally less than 3.0%. The results were considered negative if less than 4.9%, equivocal if between 5.0-9.9%, and positive if more than 10%. If dose response relationships were not observed, additional experiments were carried out at similar dose levels. The maximum dose for positive results represents the dose at which the maximum effect was obtained. For quantitative evaluation of the clastogenic potential, the D20 was calculated, which is the dose (mg/ml) at which structural aberrations (including gaps) were detected in 20% of the metaphases observed. In addition, the TR value was calculated, which indicates the frequency of cells with exchange-type aberrations per unit dose (mg/ml). These values are relatively high for chemicals that show carcinogenic potential in animals.
Result	The test substance was shown to be positive (20% total incidence of cells with aberrations) in chromosomal aberration test at 48 hours. TR value was 1.8 and D20=2.0. It was also positive at 2.0 mg/ml at 24 hour and 48 hour, (23.0 and 18%, total incidence of cells with aberrations) The results were considered positive if the total incidence of cells with aberrations (including gaps) was 10.0% or more.
Cytotoxic concentration	Not given
Genotoxic effects	Positive
Appropriate statistical evaluations?	None given
Remarks for results	Positive
Conclusion remarks	Sunset Yellow tested positive in the chromosomal aberration test using Chinese hamster fibroblasts.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report which meets basic scientific principles.
References	Ishidate, M., Sofuni, T., Yoshikawa, K., Hauashi, M., Nohmi, T., Sawada, M. and Matsuoka. (1984). Primary Mutagenicity Screening of Food Additives Currently Used in Japan. Fd.

Chem. Toxic. 22(8) 623-636.

4.2.2 In vivo Genotoxicity

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Method/guideline	Rodent Micronucleus Test
Test Type	Rodent Micronucleus
GLP	Ambiguous
Year	1991
Species/Strain	Rat/PVG
Sex	Male
Route of administration	Oral-Gavage
Doses/concentration levels	10 ml/kg bw
Exposure period	Single dose
Remarks for test conditions	Male PVG rats received a single oral dose of 500, or 1000 mg/kg of the test substance. Bone marrow samples were taken at 24 and 48 hours later.
Effect on mitotic index or PCE/NCE ratio by dose level and sex	
Genotoxic effects	No significant increase in the frequency of micronucleated polychromatic erythrocytes at either time point and in either species was reported. Additionally, there was reported increase in the % PE (polychromatic erthyrocytes).
NOEL (C)/ LOEL (C)	
Appropriate statistical evaluations?	Yes.
Remarks for results	No effects.
Conclusion remarks	

Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report which meets basic scientific principles.
References	Westmoreland C. and Gatehouse D.G. (1991) The differential clastogenicity of Solvent Yellow 14 and FD & C Yellow No. 6 in vivo in the rodent micronucleus test (observations on species and tissue specificity). Carcinogenesis 12 (8), 1403-8.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for structurally related substance, C.I. Acid Yellow 23, 94% purity
Method/guideline	Mirsalis and Butterworth, 1980
Test Type	Unscheduled DNA Synthesis
GLP	Ambiguous
Year	1985
Species/Strain	Rat/Sprague Dawley
Sex	Male
Route of administration	Oral-Gavage
Doses/concentration levels	500 mg/kg bw
Exposure period	2 hour; 15 hour
Remarks for test conditions	Six to eight male Sprague-Dawley rats weighing 200-300 g were administered 500 mg acid yellow 23/kg bw via gavage. The control animal was administered corn oil only. Animals were killed at two timepoints, 2 hr and 15 hr. If negative results were obtained at timepoint 1 and timepoint 2, the in vivo testing was terminated and considered to be negative. If the initial test at timepoint 1 yielded a positive response, the test substance was retested at that timepoint. If another positive response was observed, the test was considered positive. Timepoints are the time the test substance was administered prior to the start of liver perfusion and isolation of hepatocytes. Hepatocytes from rats were isolated and cultured according to the two step in situ liver perfusion model (Malansky and Williams, 1982). Viable hepatocytes (2 X 10+5) were seeded in wells and incubated for 4 hours with [H3]-thymidine (10 uCi/ml) and the test substance (prepared in either DMSO or water) according to a procedure similar to Williams, 1977. Control incubations were conducted with and without DMSO. The authors state that DMSO had no effect on DNA repair. DNA repair was quantified by the autoradiographic determination of incorporated [3H]-thymidine. Net nuclear grains (NNG) were determined by counting the number of

	grains in each nuclei and subtracting the average number of grains present in the three equal size adjacent cytoplasmic areas. Average NNG counts of 5 or more were assumed to constitute a positive response, because these differed from the control response by greater than 2 standard deviations. In the negative controls, NNG counts ranged from -0.6- to -2.8 and from -0.9 to -2.1 for no solvent and 1% DMSO incubations, respectively. The proportion of cells with greater than or equal to 5 NNG was less than or equal to 8.1% for all control incubations. Therefore NNG below zero were considered negative responses. Concentrations of dyes producing 90% or greater detachment of the hepatocytes from the coverslips were assumed to be toxic and not counted. The positive control was Solvent Yellow 3 (o-aminoazotoluene).
Effect on mitotic index or PCE/NCE ratio by dose level and sex	Experiment 1 Dose (mg/kg bw) Time Avg NNG % >5NNG 500 2 hr -2.6 (+/-3.7) 2 15 hr -1.3 (+/-2.6) 2
Genotoxic effects	Negative
NOEL (C)/ LOEL (C)	Greater than 500 mg/kg bw
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	C.I. Acid Yellow 23 did not induce unscheduled DNA synthesis in an *in vivo* assay using rat hepatocytes isolated from the livers of Sprague Dawley rats.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Kornbrust D. and Barfknecht T. (1985) Testing Dyes in HPC/DR systems. Environmental Mutagenesis 7, 101-120.

4.3 REPEATED DOSE TOXICITY

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow

Remarks for Substance	91.9% purity; 5.05% water; 2.77% sodium chloride
Method/guideline	National Toxicology Program. Carcinogenesis bioassay NTP 80-33
GLP	Yes
Year	1981
Species/Strain	Rats/F344/N
Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 12,500 or 25,000 ppm
Exposure period	103 weeks
Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	1 week
Remarks for test conditions	Groups of fifty male and fifty female rats each were administered 12,500 or 50,000 ppm FD & C Yellow No. 6 in the diet daily for 103 weeks. Ninety male and female rats each served as concurrent controls. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed at least monthly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder.
NOAEL(NOEL)	25,000 ppm (females); 12,500 ppm (males)
LOAEL(LOEL)	Greater than 25,000 ppm (females); 25,000 ppm (males)
Actual dose received by dose level and sex	not determined
Toxic response/effects by dose level	The mean body weights of male rats administered the high dose were slightly lower than the control animals throughout the study. The survival of male and female rats was similar between treated animals and controls (males: control 70/90 (78%); low dose 36/50 (72%); and high dose 38/50 (76%) and females: control 66/88 (75%); low dose 40/50 (80%) and high dose 37/50 (74%)). Histopathological examination revealed no evidence of carcinogenicity related to treatment with the test material. No other effects were reported.
Appropriate statistical evaluations?	Yes, Cox and Taron

Remarks for results	See Toxic response/effects by dose level.
Conclusion remarks	The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD & C Yellow No. 6 in F344/N rats.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	91.9% purity; 5.05% water; 2.77% sodium chloride
Method/guideline	National Toxicology Program. Carcinogenesis bioassay NTP 80-33
GLP	Yes
Year	1981
Species/Strain	Mice/B6C3F1
Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 12,500 or 25,000 ppm
Exposure period	103 weeks
Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	1 week (female mice)
Remarks for test conditions	Groups of fifty male and fifty female mice each were administered 12,500 or 50,000 ppm FD & C Yellow No. 6 in the diet daily for 103 weeks. Fifty male and female mice each served as concurrent controls. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed at least monthly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder.
NOAEL(NOEL)	12,500 ppm

LOAEL(LOEL)	25,000 ppm
Actual dose received by dose level and sex	not determined
Toxic response/effects by dose level	The mean body weights of male and female mice administered the high dose were slightly lower than the control animals throughout most of the study. The survival of male and female mice was similar between treated animals and controls (males: control 38/50 (76%); low dose 40/50 (80%); and high dose 33/50 (66%) and females: control 38/50 (76%); low dose 35/50 (70%) and high dose 43/50 (86%)). An increased incidence in hepatocellular carcinomas was reported among males in the low (46%) and high (32%) dose groups compared to the control males (26%), but was only a significant difference in the low dose mice. No significant differences were observed in the female animals. The increased incidence in hepatocellular carcinomas reported for male mice was not considered clearly related to administration of the test material given the variability in tumour occurrence in control male B6C3F1 mice and because the incidence of these tumours was not significantly increased in the high dose male mice.
Appropriate statistical evaluations?	Yes, Cox and Taron
Remarks for results	
Conclusion remarks	The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD & C Yellow No. 6 in B6C3F1 mice.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	91.9% purity; 5.05% water; 2.77% sodium chloride
Method/guideline	12 week range finding study. National Toxicology Program. Carcinogenesis bioassay NTP 80-33
GLP	Yes
Year	1981
Species/Strain	Rat/F344/N
Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm

Exposure period	12 weeks
Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	1 week
Remarks for test conditions	Groups of ten male and ten female rats each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD & C Yellow No. 6 in the diet daily for 12 weeks followed by one week of control diet only. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals.
NOAEL(NOEL)	6000 ppm (females); 12,500 ppm (males)
LOAEL(LOEL)	12,500 ppm (females); 25,000 ppm (males)
Actual dose received by dose level and sex	not determined
Toxic response/effects by dose level	No animals died during the study. Decreases in mean body weight gain were reported for male rats at the 25,000, 50,000 or 100,000 ppm intake levels. For female rats, decreases in mean body weight gain were reported at the 12,500, 25,000, 50,000 or 100,000 ppm intake levels. Bone marrow hyperplasia was reported in all examined animals at the 50,000 or 100,000 ppm intake levels.
Appropriate statistical evaluations?	Yes, Cox and Taron
Remarks for results	See Toxic response/effects by dose level.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	91.9% purity; 5.05% water; 2.77% sodium chloride
Method/guideline	12 week range finding study. National Toxicology Program. Carcinogenesis bioassay NTP 80-33
GLP	Yes
Year	1981
Species/Strain	Mice/B6C3F1

Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm
Exposure period	12 weeks
Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	1 week
Remarks for test conditions	Groups of ten male and ten female mice each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD & C Yellow No. 6 in the diet daily for 12 weeks followed by one week of control diet only. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals.
NOAEL(NOEL)	50,000 ppm (male); less than 6000 ppm (female)
LOAEL(LOEL)	100,000 ppm (male); 6000 ppm (female)
Actual dose received by dose level and sex	not determined
Toxic response/effects by dose level	Mean body weight gain was decreased compared to controls among male mice receiving the 100,000 ppm intake level. Decreases in body weight gain were also reported for female mice at all intake levels, and was dose related from 12,500 ppm to 100,000 ppm. Gross and histopathological examinations revealed no treatment related lesions in male or female mice at any intake level.
Appropriate statistical evaluations?	Yes, Cox and Taron
Remarks for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.

4.4 DEVELOPMENTAL TOXICITY

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Method/guideline	Teratogenicity study
Test Type	
GLP	Ambiguous
Year	1974
Species/Strain	Rat/Charles River CD
Sex	Female
Route of administration	Oral-Gavage
Duration of test	20 days
Doses/concentration levels	0, 100, 300 or 1000 mg/kg bw/day
Exposure period	9 days (6-15 of gestation)
Frequency of treatment	Daily
Control Group and treatment	Yes, three negative control groups were maintained and administered 0.5% methocel, while one positive control group was maintained and administered 7.5% mg/kg bw/day of retinoic acid.
Remarks for test conditions	FD&C Yellow No. 6 was administered by gavage at dose levels of 100, 300 or 1000 mg/kg bw/day to 140 female Charles River CD rats. Three negative control groups (20/group) received the vehicle control while one control group received the positive control (7.5% mg/kg bw/day retinoic acid). All females were dosed on days 6-15 of gestation. Cesarean sections were performed on the 20th day of gestation.
NOAEL(NOEL) maternal toxicity	
LOAEL(LOEL) maternal toxicity	Not given
NOAEL (NOEL) developmental toxicity	100 mg/kg bw/day (based on decreased mean fetal weights in one of three control groups)
LOAEL (LOEL) developmental toxicity	300 mg/kg bw/day
Actual dose received by dose level and sex	Not given
Maternal data with dose level	Mean body weights for dams in control groups were not statistically different from any of the test groups of dams nor from the mean body weights of the positive control group (7.5 mg/kg/ bw/d retinoic acid).
Fetal data with dose level	The mean weights of the offspring from the 300 and 1000 mg/kg bw/day groups were decreased when compared to the

mean fetal weight of one of the three negative controls. However, there difference in mean body weight of the 300 and 1000 mg/kg bw/d groups was not statistically different from the combined negative control group mean. There were no compound related effects on early or late resorptions, empty implantation sites, body weight or numbers of live or dead fetuses. There was a statistical increase in the number of abnormal young in the positive control group. No teratogenicity was observed among the offspring exposed to Yellow No. 6.

Group	No. corpora lutes	No implantation sites
Veh 1	279	230
Veh 2	296	265
Veh 3	276	275
Retinoic	269	265
100 mg/kg	297	255
300 mg/kg	301	252
1000 mg/kg	291	283

Group	No empty implantation sites	No. resorptions	No dams w/resorptions
Veh 1	9	4	2
Veh 2	17	0	0
Veh 3	7	1	1
Retinoic	15	1	1
100 mg/kg	20	1	1
300 mg/kg	7	2	2
1000 mg/kg	16	0	0

Group	No normal young		No. abnormal young		No fetuses aborted
	alive	dead	alive	dead	
Veh 1	200	0	17	0	0
Veh 2	217	0	31	0	0
Veh 3	212	0	55	0	0
Retinoic	91	0	158	0	0
100 mg/kg	210	0	24	0	0
300 mg/kg	219	0	24	0	0
1000 mg/kg	236	0	31	0	0

Appropriate statistical evaluations?

Yes
Dunnett C.W. (1964) New tables for multiple comparisons with a control, Biometrics
Steel and Torrie (1960) Principles and procedures of statistics, McGraw-Hill, New York, NY.

Remarks for results

Based on the result of the study Yellow No. 5 exhibits no teratogenic potential.

Conclusion remarks

Data Qualities Reliabilities

Reliability code 2. Reliable with restriction.

Remarks for Data Reliability

Code 2. Basic data given: comparable to guidelines/standards.

References

International Research and Development Corporation (1972) Teratology study in rats. Compound FD&C Yellow No. 6. Unpublished report no. 306-004.

4.5 REPRODUCTIVE TOXICITY

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Method/guideline	Long-Term In-Utero study in male and female rats
Test Type	Long-Term In-Utero Study in Rats
GLP	Yes
Year	1981
Species/Strain	Rat/Charles River Albino (CD) (R)
Sex	Male and Female
Route of administration	Oral-Diet
Duration of test	Duration of Feeding (F0 Generation) - 126 days Duration of Feeding (Fl Generation) - 901 days (males); 854 or 855 days (females)
Doses/concentration levels	0.75, 1.5, or 3.0% in the diet (calculated to provide an average daily intake of 750, 1500, and 3000 mg/kg bw per day
Premating Exposure period for males	Both sexes of the F0 generation received the test material for approximately two months prior to mating
Premating Exposure period for females	Both sexes of the F0 generation received the test material for approximately two months prior to mating
Frequency of treatment	Daily
Control Group and treatment	Yes (Two control groups each for males and females).
Remarks for test conditions	Control and Fo generation- 60 animals/sex/group Control and F1 generation -70 animals/sex/group Mating and Reproductive Phase - After the F0 generation received the test material for approximately two months, males and females were housed together in a 1:1 ratio for a one week mating period. After gestation and a 21-day lactation period, pups were weaned and remained together for 13 to 19 days until selection of F1 animals. Animals were housed individually in elevated stainless steel cages, except during mating, lactation and post-weaning phases. Water was provided ad libitum by an automated water system. Animals were maintained on a 12-hour light/dark cycle.

Temperature and humidity were monitored twice daily. Desired temperature and humidity ranges were 68-76"F and 40-60%.

A sample of each lot of feed was forwarded to Raltech Scientific Services, Madison Wisconsin 53707 for analyses. Samples of control and of each test diet were taken weekly throughout the study. Samples were assayed for batch homogeneity at week 1 when a twin-shell mixer was used and at week 61 when a rotary mixer was used for preparation of the diets. Storage stability of samples at room temperature and at 37 C was determined at 0, 7, 14, and 24 days. Samples were assayed for concentration of test substance weekly for the first 13 weeks then at week 16 and every 4 weeks thereafter to week 148 inclusive. Feed samples were also taken at the beginning and end of weeks 4, 8, 12, and 16 and assayed for concentration of test substance.

Bodyweights, food consumption, hematology and clinical chemistry parameters, absolute and relative organ weights, survivorship, and tumor incidence data were statistically analyzed.

For the Fo generation the following parameters were monitored:
General Appearance and Behavior - Twice daily
Survival - Twice daily
Detailed Physical Examination – Weekly
Ophthalmoscopic Examination - Pretest
Body Weight-
Males: Twice pretest and weekly during the premating and mating periods Females: Twice pretest and weekly during the premating, mating, and gestation periods and on days 0, 4, 14, and 21 of lactation
Food Consumption:
Males: Pretest, and weekly during the premating period
Females: Pretest, weekly during the premating period and for the first two weeks of gestation

Number of Pregnant Females/Group
Gross Pathology - Sacrificed post-weaning; no necropsy performed. Animals dying spontaneously or killed in moribund condition were given a complete gross postmortem examination.
For pups:
Viability at days 0, 4, 14, and 21
Mean Body Weight at days 0, 4, 14, and 21
Post-weaning Survival

For F1 Generation:
General Appearance and Behavior - Twice daily
Survival - Twice daily
Detailed Physical Examination - Weekly
Ophthalmoscopic Examination - Initial and at months 3, 6, 12, 18, and 24
Body weights- Initial, weekly through 13 weeks, biweekly 14 through 26 weeks, approximately monthly thereafter and

	terminally (after fasting). Individual and mean body weights were furnished at weeks -1 (initial weights following random selection), 1, 4, 8, 13, 26, 51, 76 and i00 for both sexes and at week 120 for females and at week 128 for males. Food Consumption -Data were obtained and furnished for same intervals as body weights. Laboratory Studies-On 10 rats/sex/group at months 3, 6, 12, 18 and 24. Hematology–Hemoglobin, hematocrit, erythrocyte counts, total and differential leukocyte counts, and erythrocyte morphology Clinical Chemistry-Serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, blood urea nitrogen, fasting blood glucose, total protein, and creatinine Urinanalysis- Gross appearance, specific gravity, pH, protein, glucose, ketches, bilirubin, occult blood, and microscopic analysis Gross pathology: Animals dying spontaneously or killed in moribund condition were given a complete gross postmortem examination. Necrospy was performed on 10 rats/sex/group sacrificed at month 12 and on all survivors at termination of the study. Animals were sacrificed by exsanguination under ether anesthesia Organ weights-Individual and mean terminal body weights and absolute and relative weights of brain, gonads, thyroids, spleen, kidneys, and liver of I0 rats/sex/group sacrificed at month 12 and of all antis sacrificed at termination of study Histopathology-Tissues from all organs weighed plus about 28 other tissues were examined microscopically from i0 rats/sex/group from each control group and from the high dietary level group at the interim sacrifice and from all survivors from these groups at termination as well as of any animal dying spontaneously or sacrificed in extremis from these groups. In addition, microscopic examination of tissues exhibiting gross changes of uncertain nature and of all tissue masses was performed for all animals.
NOAEL(NOEL)	1500 mg/kg bw/day for the Fo, pups, and F1 generation
LOAEL(LOEL)	Not determined
Actual dose received by dose level and sex	Not given
Parental data and F1 as appropriate	See below
Offspring toxicity F1 and F2	See below
Appropriate statistical evaluations?	Yes
Remarks for results	For the Fo generation- No effect on general appearance and behavior and survival of the rats was noted. During the premating period of approximately two months, mean body weights were lower and mean food consumption was elevated for the males on the 3.0 and 1.5% dietary levels; these differences from the control

values were reported by the performing laboratory to be dose-related and statistically significant. No dose-related effect on the number of pregnant females/group was observed.

For the Pups-
Mean pup weight at birth was slightly greater for the 3.0% dietary level group than for the control groups. Pup viability was reduced for this high dietary level group at Day 4 and Day 21 of lactation. The 3.0% dietary level group had the lowest mean pup weight at day 21 of lactation. Mean pup weight was also reduced for the 1.5% dietary level group at day 21 of lactation. All other criteria evaluated including post-weaning survival were comparable for the control and treated groups of pups.

For the F1 generation-
No adverse effects on general appearance and behavior of the rats were noted. Mortality was slightly increased in f6m~les on the 3.0% dietary level of FD&C Yellow No. 6 from 25 through 29 months of feeding compared to the female controls; however, the difference was not statistically significant. When survival reached nine animals of the same sex on the 3.0% dietary level of the color additive, all surviving animals of that sex were sacrificed. This occurred during week 122 for females and week 129 for males. Ocular abnormalities seen during the study were not attributable to administration of the test compound.
Mean body weights for the groups of rats on the 3.0 and 1.5% dietary levels were lower than those of the control groups of rats at initiation of the study, consistent with the lower mean pup weights for these groups prior to random selection of offspring for the FI generation. Thereafter, mean body weights of the treated and control groups were generally comparable for most of the remainder of the study. Mean body nights of the females on the 3.0% dietary level were lower than those of the control females from week 100 of the study. At week 128, mean body ~eights of the males on the 3.0 and 1.5% dietary levels were lower than those of the control males. The differences from control noted late in the study were not statistically significant, although they were as much as 10% and 8% below control values for the females and males, respectively. Statistically significant, dose-related, increased mean food consumption values for the treated groups compared to the control groups of rats were recorded during the first 26 weeks and four weeks of the study for the males and females, respectively. Statistically significant increased mean food consumption values for the 3.0% dietary level of males at 51 and 78 weeks and females at 8, 13, 26, 76, and I00 weeks and for the 1.5% dietary level males at 78 weeks and females at 13, 26, and 76 weeks were reported.

There were no consistent trends in the mean hematology values of the treated and pooled control groups that would suggest any relationship to treatment. Elevated, statistically significant mean blood urea nitrogen values were found in the 3.0% dietary level female rats at months 18 and 24 compared to the pooled control females. Slight elevations in serum

glutamic oxaloacetic transaminase activity noted in the male rats on the 3.0 and 1.5% dietary levels at months 18 and 24 were not statistically significant and were not considered to be toxicologically significant for aged male rats. Urine samples from the treated animals were generally yellow or amber to orange in appearance, whereas control urine samples were normal, i.e., straw to yellow colored in appearance. Individual urinalysis values for the treated and control animals were comparable.

Organ weights, gross and microscopic examination of the tissues of the rats through 12 months of the study revealed no morphologic evidence of any adverse effect related to dietary feeding of FD&C Yellow No. 6. Gross postmortem examinations of the rats fed FD&C Yellow No. 6 after the 12 month interim sacrifice and before the terminal sacrifice revealed a pigmented gastrointestinal tract described as orange, yellow, or yellow-green in 10/147 males and 14/133 females.

For the female rats on the 3.0% dietary level of FD&C Yellow No. 6 compared to the pooled control groups of female rats sacrificed at termination of the study, both mean absolute and relative kidney weights were increased. Only the increase in mean relative weight of the kidneys was reported to be statistically significant. The increase in absolute kidney weights in spite of the decrease in mean body weight might indicate the kidneys were enlarged in this test group.

Histopathological examination of the rats through termination of the study revealed increased incidences of female rats with adrenal medullary adenoma (13/69 or 18.8%) on the 3.0% dietary level compared to the incidence of control females with the lesions (10/139 or 7.2%). Because of the increased incidence of the adrenal lesions seen in this study in the 3.0% dietary level females and in the 5.0% dietary level females of the high dose study NTP study, the test laboratory resectioned the adrenals and reexamined the adrenal microslides of females in the two studies. These slides were also examined by FDA/CFSAN pathologists. On the basis of the pathologist's findings and other considerations, the Cancer Assessment Committee concluded that the increases in the number of female rats with adrenal medullary lesions is unrelated to treatment with FD&C Yellow No. 6 (FDA, December 3, 1985).

Histopathological examination also revealed an increased incidence of rats with testicular interstitial cell adenoma in the group on the 3.0% dietary level (15/70 or 21.4%) compared to the incidence (14/138 or 10.1%) in the pooled control groups. The incidence for the treated group is near the maximum control incidence reported by Bio/dynamics Inc., in this rat strain. This was in the study of FD&C Blue No. 2 where the reported incidence of testicular interstitial cell adenoma was 27/137 or 19.7% for the contemporary pooled control groups. It was concluded that the increased incidence of rat testicular interstitial ceil adenomas was not treatment-related because

	the rats on the 5.0% dietary level in the NTP chronic study did not show an increased incidence. The incidences of testicular tumors were concluded to be unrelated to administration of the test vehicle.
Conclusion remarks	The result of the long-term in utero study in male and female rats show not significant evidence of reproductive toxicity related to the intake of Yellow No. 6
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Equivalent to a standardized guideline study but of longer duration.
References	Bio/dynamics Inc. (1981) First Long-Term In-Utero Study in Rats (Study No. 77-1778) .

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Method/guideline	Long-Term In-Utero study in male and female rats
Test Type	Long-Term In-Utero Study in Rats
GLP	Yes
Year	1982
Species/Strain	Rat/Charles River Albino (CD) (R)
Sex	Male and Female
Route of administration	Oral-Diet
Duration of test	Age of F_0 generation at start -50-57 days Duration of Feeding (F_0 Generation) - 125 days Duration of Feeding (F_I Generation) - 767 days (males); 834 days (females
Doses/concentration levels	0 or 5.0% in the diet (calculated to provide an average daily intake of 0 or 5000 mg/kg bw per day
Premating Exposure period for males	Both sexes of the F_0 generation received the test material for approximately two months prior to mating
Premating Exposure period for females	Both sexes of the F_0 generation received the test material for approximately two months prior to mating
Frequency of treatment	Daily
Control Group and treatment	Yes (Two control groups each for males and females.

Remarks for test conditions

Control and Fo generation- 60 animals/sex/group
Control and F1 generation -70 animals/sex/group

Mating and Reproductive Phase - After the F_0 generation received the test material for approximately two months, males and females were housed together in a 1:1 ratio for a one week mating period. After gestation and a 21-day lactation period, pups were weaned and remained together for 13 to 19 days until selection of F_1 animals.

Animals were housed individually in elevated stainless steel cages, except during mating, lactation and post-weaning phases. Water was provided ad libitum by an automated water system. Animals were maintained on a 12-hour light/dark cycle. Temperature and humidity were monitored twice daily. Desired temperature and humidity ranges were 68-76"F and 40-60%.

A sample of each lot of feed was forwarded to Raltech Scientific Services, Madison Wisconsin 53707 for analyses. Samples of control and of each test diet were taken weekly throughout the study. Samples were assayed for batch homogeneity at week 1 when a twin-shell mixer was used and at week 61 when a rotary mixer was used for preparation of the diets. Storage stability of samples at room temperature and at 37 C was determined at 0, 7, 14, and 24 days. Samples were assayed for concentration of test substance weekly for the first 13 weeks then at week 16 and every 4 weeks thereafter to week 148 inclusive. Feed samples were also taken at the beginning and end of weeks 4, 8, 12, and 16 and assayed for concentration of test substance.

Bodyweights, food consumption, hematology and clinical chemistry parameters, absolute and relative organ weights, survivorship, and tumor incidence data were statistically analyzed. Body weight measurements for the F_1 generation were initial, weekly through 14 weeks, biweekly 16 through 26 weeks, monthly thereafter and terminally (after fasting). Individual and mean body weights were reported at weeks -1 (initial weights following random selection), 1, 5, 8, 13, 26, 50, 78, and 102 for both sexes and at week 106 for males and at week 118 for females

For the Fo generation the following parameters were monitored:
General Appearance and Behavior - Twice daily
Survival - Twice daily
Detailed Physical Examination – Weekly
Ophthalmoscopic Examination - Pretest
Body Weight-
Males: Twice pretest and weekly during the premating and mating periods Females: Twice pretest and weekly during the premating, mating, and gestation periods and on days 0, 4, 14, and 21 of lactation
Food Consumption:
Males: Pretest, and weekly during the premating period

Females: Pretest, weekly during the premating period and for the first two weeks of gestation

Number of Pregnant Females/Group
Gross Pathology - Sacrificed post-weaning; no necropsy performed. Animals dying spontaneously or killed in moribund condition were given a complete gross postmortem examination.
For pups:
Viability at days 0, 4, 14, and 21
Mean Body Weight at days 0, 4, 14, and 21
Post-weaning Survival

For F1 Generation:
General Appearance and Behavior - Twice daily
Survival - Twice daily
Detailed Physical Examination - Weekly
Ophthalmoscopic Examination - Initial and at months 3, 6, 12, 18, and 24
Body weights- Initial, weekly through 13 weeks, biweekly 14 through 26 weeks, approximately monthly thereafter and terminally (after fasting). Individual and mean body weights were furnished at weeks -1 (initial weights following random selection), 1, 4, 8, 13, 26, 51, 76 and i00 for both sexes and at week 120 for females and at week 128 for males.
Food Consumption -Data were obtained and furnished for same intervals as body weights.
Laboratory Studies-On 10 rats/sex/group at months 3, 6, 12, 18 and 24.
Hematology–Hemoglobin, hematocrit, erythrocyte counts, total and differential leukocyte counts, and erythrocyte morphology
Clinical Chemistry-Serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, blood urea nitrogen, fasting blood glucose, total protein, and creatinine
Urinanalysis- Gross appearance, specific gravity, pH, protein, glucose, ketches, bilirubin, occult blood, and microscopic analysis
Gross pathology: Animals dying spontaneously or killed in moribund condition were given a complete gross postmortem examination. Necrospy was performed on 10 rats/sex/group sacrificed at month 12 and on all survivors at termination of the study. Animals were sacrificed by exsanguination under ether anesthesia
Organ weights-Individual and mean terminal body weights and absolute and relative weights of brain, gonads, thyroids, spleen, kidneys, and liver of I0 rats/sex/group sacrificed at month 12 and of all antis sacrificed at termination of study
Histopathology-Tissues from all organs weighed plus about 28 other tissues were examined microscopically from i0 rats/sex/group from each control group and from the high dietary level group at the interim sacrifice and from all survivors from these groups at termination as well as of any animal dying spontaneously or sacrificed in extremis from these groups. In addition, microscopic examination of tissues exhibiting gross changes of uncertain nature and of all tissue masses was

	performed for all animals.
NOAEL(NOEL)	Not determined
LOAEL(LOEL)	Not determined
Actual dose received by dose level and sex	Not given
Parental data and F1 as appropriate	See below
Offspring toxicity F1 and F2	See below
Appropriate statistical evaluations?	Yes
Remarks for results	For the Fo generation- During treatment for a minimum of eight weeks prior to initiation of the mating period, no effect on general appearance and behavior and survival of the rats was noted. However, compared to the control animals, mean body weights were lower for the males and mean food consumption was elevated for the males and females on the 5.0% dietary level; these differences from the control values were reported by the performing laboratory to be generally statistically significant. No treatment-related effect on the number of pregnant females/group was observed. For the Pups- Mean pup weight and viability at birth were comparable for the control and treated groups. Pup viability for the 5.0% dietary level group was lower than that of the control group during the day 0 to 14 interval of lactation and during the post-weaning period. Mean pup weight of the treated group was lower than that of the control group at day 21 of lactation. For the F1 generation- No adverse effects on general appearance and behavior of the rats were noted. Mortality was slightly increased in f6m~les on the 3.0% dietary level of FD&C Yellow No. 6 from 25 through 29 months of feeding compared to the female controls; however, the difference was not statistically significant. When survival reached nine animals of the same sex on the 3.0% dietary level of the color additive, all surviving animals of that sex were sacrificed. This occurred during week 122 for females and week 129 for males. Ocular abnormalities seen during the study were not attributable to administration of the test compound. Mean body weights for the groups of rats on the 3.0 and 1.5% dietary levels were lower than those of the control groups of rats at initiation of the study, consistent with the lower mean pup weights for these groups prior to random selection of offspring for the F_l generation. Thereafter, mean body weights of the treated and control groups were generally comparable for most of the remainder of the study. Mean body nights of the females on the 3.0% dietary level were lower than those of the control females from week 100 of the study. At week 128, mean

body ~eights of the males on the 3.0 and 1.5% dietary levels were lower than those of the control males. The differences from control noted late in the study were not statistically significant, although they were as much as 10% and 8% below control values for the females and males, respectively. Statistically significant, dose-related, increased mean food consumption values for the treated groups compared to the control groups of rats were recorded during the first 26 weeks and four weeks of the study for the males and females, respectively. Statistically significant increased mean food consumption values for the 3.0% dietary level of males at 51 and 78 weeks and females at 8, 13, 26, 76, and I00 weeks and for the 1.5% dietary level males at 78 weeks and females at 13, 26, and 76 weeks were reported.

There were no consistent trends in the mean hematology values of the treated and pooled control groups that would suggest any relationship to treatment. Elevated, statistically significant mean blood urea nitrogen values were found in the 3.0% dietary level female rats at months 18 and 24 compared to the pooled control females. Slight elevations in serum glutamic oxaloacetic transaminase activity noted in the male rats on the 3.0 and 1.5% dietary levels at months 18 and 24 were not statistically significant and were not considered to be toxicologically significant for aged male rats. Urine samples from the treated animals were generally yellow or amber to orange in appearance, whereas control urine samples were normal, i.e., straw to yellow colored in appearance. Individual urinalysis values for the treated and control animals were comparable.

Organ weights, gross and microscopic examination of the tissues of the rats through 12 months of the study revealed no morphologic evidence of any adverse effect related to dietary feeding of FD&C Yellow No. 6. Gross postmortem examinations of the rats fed FD&C Yellow No. 6 after the 12 month interim sacrifice and before the terminal sacrifice revealed a pigmented gastrointestinal tract described as orange, yellow, or yellow-green in 10/147 males and 14/133 females.

For the female rats on the 3.0% dietary level of FD&C Yellow No. 6 compared to the pooled control groups of female rats sacrificed at termination of the study, both mean absolute and relative kidney weights were increased. Only the increase in mean relative weight of the kidneys was reported to be statistically significant. The increase in absolute kidney weights in spite of the decrease in mean body weight might indicate the kidneys were enlarged in this test group.

Histopathological examination of the rats through termination of the study revealed increased incidences of female rats with adrenal medullary adenoma (13/69 or 18.8%) on the 3.0% dietary level compared to the incidence of control females with the lesions (10/139 or 7.2%). Because of the increased incidence of the adrenal lesions seen in this study in the 3.0%

dietary level females and in the 5.0% dietary level females of the high dose study NTP study, the test laboratory resectioned the adrenals and reexamined the adrenal microslides of females in the two studies. These slides were also examined by FDA/CFSAN pathologists. On the basis of the pathologist's findings and other considerations, the Cancer Assessment Committee concluded that the increases in the number of female rats with adrenal medullary lesions is unrelated to treatment with FD&C Yellow No. 6 (FDA, December 3, 1985).

Histopathological examination also revealed an increased incidence of rats with testicular interstitial cell adenoma in the group on the 3.0% dietary level (15/70 or 21.4%) compared to the incidence (14/138 or 10.1%) in the pooled control groups. The incidence for the treated group is near the maximum control incidence reported by Bio/dynamics Inc., in this rat strain. This was in the study of FD&C Blue No. 2 where the reported incidence of testicular interstitial cell adenoma was 27/137 or 19.7% for the contemporary pooled control groups. It was concluded that the increased incidence of rat testicular interstitial ceil adenomas was not treatment-related because the rats on the 5.0% dietary level in the NTP chronic study did not show an increased incidence. The incidences of testicular tumors was concluded to be unrelated to administration of the test vehicle.

Conclusion remarks	The result of the long-term in utero study in male and female rats show not significant evidence of reproductive toxicity related to the intake of Yellow No. 6
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Equivalent to a standardized guideline study but of longer duration.
References	Bio/dynamics Inc. (1982) Additional Long-Term In-Utero Study in Rats (Study No. 78-2211) .

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Method/guideline	3-generation reproductive study
Test Type	
GLP	Ambiguous
Year	1974
Species/Strain	Rat/Charles River CD

Sex	Male and Female
Route of administration	Oral-Diet
Duration of test	
Doses/concentration levels	5, 50, 150 or 500 mg/kg bw/day
Premating Exposure period for males	Approximately 2 months
Premating Exposure period for females	Approximately 2 months
Frequency of treatment	Daily in the diet
Control Group and treatment	Yes.
Remarks for test conditions	One hundred twenty Charles River CD rats (10 males and 20 females/group/generation) received 5, 50, 150 or 500 mg/kg bw/day of the test substance as a dietary admixture in a three-generation study. Ten males and twenty females received no compound and served as controls. Animals were housed individually and fed the test diet ad libitum. Clinical observations were recorded twice daily with at least 5 hours between observations. Detailed physical examinations and palpation for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for the next 12 weeks and every 4 weeks thereafter until the end of the study. The intake of the test substance was determined from body weight, food consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on ten randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (2.0 or 5.0%) from each study and also on 10 rats randomly selected from each group for an interim sacrifice at 12 months. Histology was also performed on any animal with gross lesions or masses. Tissues examined included adrenal glands, aorta, blood smear, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, duodenum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes with epididymides, stomach, thymus, thyroid gland including parathyroid, trachea, urinary bladder, uterus.
NOAEL(NOEL)	500 mg/kg bw/day
LOAEL(LOEL)	Not determined
Actual dose received by dose level and sex	Not given

Parental data and F1 as appropriate	There was no toxicity to either the parent or F1 generation
Offspring toxicity F1 and F2	There was no toxicity to either the F1 or F2 generation. Food consumption was similar for control and treated animals at the lower dietary levels, but was slightly higher in the high-dose study, although not statistically significant. Hematological, clinical chemistry and urinalysis parameters did not differ significantly from the controls. Necropsies at one year did not reveal any treatment-related gross or microscopic changes. At study termination, no treatment-related effects were reported on survival. No treatment-related changes were reported at gross necropsy. Histological evaluation revealed a variety of lesions, including neoplasms, present at similar incidences in control and treated animals. The authors considered the lesions to be spontaneous and not related to administration of the test material.
Appropriate statistical evaluations?	Yes
Remarks for results	There were no compound related effects on fertility, gestation, pup viability or lactation indices, on reproductive organs of females, or on organ weights among parents and offspring. There were no compound related lesions in any tissue examined histologically, including kidneys and adrenal glands from parental rats or from offspring.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	International Research and Development Corporation (1974) Multi-generation reproduction study in rats. Compound FD&C Yellow No. 6. Unpublished report no. 306-005.

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EPA Comments on Chemical RTK HPV Challenge Submission:
Sunset Yellow

Summary of EPA Comments

The sponsor, the International Association of Color Manufacturers (IACM), submitted a test plan and robust summaries to EPA for Sunset Yellow (FD&C Yellow No. 6; C.I. Food Yellow No. 3; CAS No. 2783-94-0) dated March 10, 2004. EPA posted the submission on the ChemRTK HPV Challenge Web site on March 19, 2004. Information is also submitted on FD&C Red No. 40, C.I. Acid Red No. 14, stilbene sulfonic acid derivatives, and C.I. Acid Yellow 23, as analogs. [CAS Numbers for these analogs are not provided.]

EPA has reviewed this submission and has reached the following conclusions:

1. Analog Justification. EPA disagrees with the submitter's proposal to use certain other azo dyes and stilbene sulfonic acid derivatives as representative compounds for the sponsored chemical.

Response: IACM has provided ecotoxicity data for fish and daphnia for structurally related azo dyes containing naphthalenesulfonic acid or benzenesulfonic acid substituents and phenolic substituents. These data (LC50 >100 mg/l) confirm that azo dyes containing multiple sulfonic acid and other polar functional groups that exist in ionized form in vivo, are of low ecotoxic potential. Reference to a database of more than 3000 dyes and pigments supports this conclusion. The water solubility and other physiochemical properties including molecular weight and ionic nature under environmental conditions indicate that these compounds are not absorbed in vivo. The lack of absorption is reflected in the observed very low toxic potential for azo dyes.

2. Physicochemical Properties. The data submitted for these endpoints are adequate for the purposes of the HPV Challenge Program.
3. Environmental Fate. The submitter needs to provide the measured ready biodegradation data on the sponsored chemical, include technical discussion on stability in water in the robust summary, and provide the input values for parameters used in the Level III fugacity robust summary.

Response: These data have been included.

4. Health Effects. Adequate data are available for the acute, repeated-dose, and genetic toxicity endpoints for the purposes of the HPV Challenge Program. The data submitted for the reproductive toxicity endpoint are inadequate. EPA reserves judgement on the adequacy of the data submitted for developmental toxicity pending submission of critical study information. Testing is needed to address reproductive (and possibly developmental) toxicity. The submitter also needs to address deficiencies in the robust summaries.

Response: While we agree that the 3-generation reproductive toxicity study does not include a guideline level of 1000 mg/kg bw/d, the lack of any effects in a rigorous 3-generation study at 500 mg/kg bw/d provides an adequate basis for assessing the hazard potential of Sunset Yellow, a FDA approved food colorant. Because of it is added to the food supply, this substance has been the subject of other long-term in utero studies at levels in excess of 1000 mg/kg bw/d. Although no strictly reproduction studies, these long term two-generation studies provide relevant data for assessing the reproductive hazard of Sunset Yellow. The protocol for these studies is more comprehensive that are those of a guideline reproduction study. For instance, males and females were maintained on diets containing the test materials for two months pre-mating, through mating

and gestation and weanling. Offspring were then treated throughout their lifetime. The robust summaries for these studies demonstrate their comprehensive nature. The combination of the results of these two in utero studies and the 3-generation study are sufficient to assess the hazard potential of this substance.

Additional data for the developmental toxicity study have been included in the robust summary. Based on parameters measured in the offspring and the fact that there was clear evidence of developmental toxicity in the positive control group, Sunset Yellow exhibits no evidence of developmental toxicity.

5. Ecological Effects. Ecological endpoints have not been addressed adequately for the purposes of the HPV Challenge Program. The submitter needs to provide data for all endpoints on the sponsored chemical.

Response: Four studies evaluating ecotoxicity in fish and two studies evaluating the ecotoxicity in aquatic invertebrates have been included. The azo dyes used in these studies (5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methylbenzenesulfonic acid and 2-naphthalenecarboxylic acid, [(4-methyl-2-sulfophenyl)azo], calcium salt) contain functional groups (e.g., sulfonic acid and carboxylic acids) that are responsible for the limited solubility, absorption, and toxicity of FD&C Yellow No. 6, other colors and dyes. The database on more than 3000 dyes and pigments clearly demonstrates the low ecotoxic potential of benzene and naphthalene sulfonic acid azo dyes. The presence of similarly structured carbon analogs (stilbene sulfonic acid derivatives) containing sulfonic acid groups also show a similar low level of ecotoxic potential.

EPA Comments on the Sunset Yellow Challenge Submission

Analog Justification

The test plan provided analog data to address or support the direct photodegradation, biodegradation, aquatic toxicity, and *in vivo* genetic toxicity endpoints; however, it did not provide any rationale supporting these analogs.

EPA disagrees with the submitter that the stilbene sulfonic acid derivatives proposed to supply data for the acute fish and invertebrate toxicity endpoints are appropriate analogs for the sponsored chemical. All the stilbene analogs lack the –N=N– linkage, the phenol function, and the naphthalene group of the sponsored substance, and contain amino or nitro groups not present in the sponsored chemical.

Response: Data for azo dyes containing phenolic and sulfonic acid groups and naphthalene carbon skeletons have been provided appropriate ecotoxicity endpoints (fish and invertebrates). As EPA remarked for FD&C Yellow No. 5, data on aquatic plants is of limited use in that these colorants are intended to absorb in the visible region of the spectrum and will therefore, slow plant growth.

Although Acid Red 14 has some similarity to the sponsored chemical, its adequacy as an analog is moot because the cited biodegradation data are inadequate as noted below.

Test Plan

Physicochemical Properties (melting point, boiling point, vapor pressure, water solubility, and partition coefficient)

The data provided by the submitter for these endpoints are adequate for the purposes of the HPV Challenge Program.

Environmental Fate (photodegradation, stability in water, biodegradation, fugacity)

The data provided for photodegradation are adequate for the purposes of the HPV Challenge Program.

Stability in water. While EPA agrees that Sunset Yellow does not contain water-sensitive functional groups, the submitter needs to add a brief technical discussion of this point to the robust summary.

Response: This has been included in the test plan, a more appropriate vehicle for this chemical-based discussion.

Biodegradation. The biodegradation data are not adequate for the purposes of the HPV Challenge Program. The BIOWIN-estimated data are not adequate in place of measured data. The facts do not sustain the submitter's argument–based on data from a non-standard (only 24-hr) test on proposed analog Acid Red 14–that the test substance will not biodegrade because it does not adsorb to sludge. Although Acid Red 14 does not biodegrade under the conditions of the test, several other structurally related dyes mentioned in Shaul *et al.* 1991 are readily biodegradable but do not appear to adsorb to sludge under similar test conditions. The submitter needs to provide measured ready biodegradation data for Sunset Yellow following OECD TG 301.

Response: The several other structural relatives mentioned in the Shaul reference are not substituted with two sulfonic acid functional groups and therefore, are not good structural relatives. However, additional data has been provided on biodegradation on azo benzene sulfonic acid dyes containing two sulfonic acid and phenolic substituents. In all cases the model data for FD& C Yellow No. 6 and experimental data for other azo benzene and naphthalenesulfonic acid derivatives show no significant biodegradability.

Fugacity. The submitter needs to include the input values for parameters used in the Level III estimation in the robust summary.

Response: Input values have been included.

Health Effects (acute toxicity, repeated-dose toxicity, genetic toxicity, and reproductive/developmental toxicity)

Adequate data are available for the acute, repeated-dose, and genetic toxicity endpoints for the purposes of the HPV Challenge Program. The data submitted for the reproductive toxicity endpoint are inadequate. EPA reserves judgment on adequacy of the data submitted for the developmental toxicity endpoint. Testing will be needed to address the reproductive and possibly the developmental toxicity. The submitter needs to address deficiencies in the robust summaries.

Reproductive toxicity. The submitted 3-generation reproductive toxicity study in rats is not adequate. The maximum dose tested, 500 mg/kg/day, was much lower than the OECD guideline-required dose level of 1000 mg/kg/day, and no systemic toxicity was shown in the parental animals. In addition, critical information was missing from the robust summary, including the purity of the test material, the experimental design (especially the timing of exposure with respect to mating and termination), and the parental and fetal endpoints examined. A combined reproductive/developmental toxicity screening test will be needed following OECD TG 421 (see following comments).

Response: These data have been included in the robust summary. Also, two additional long-term in utero studies in which parents and pups are exposed throughout their lifetime to Sunset Yellow have been included. The weight of evidence clearly demonstrates Sunset Yellow does not exert any significant toxicity to reproductive.

Developmental toxicity. EPA was unable to determine the adequacy of the submitted teratogenicity study

in rats because of insufficient study details in the robust summary. Critical information missing included the purity of the test material and the maternal and fetal endpoints that were examined, such as the litter size, weight, and sex, number of fetuses examined for external, skeletal and visceral alterations, gravid uterine weights, number of corpora lutea, number of implantations, and statistical significance of any reported findings. The submitter needs to provide the above information to allow an independent assessment of study adequacy and the validity of the stated NOAEL and LOAEL. If the additional information is not available, a combined reproductive/developmental toxicity screening test (OECD TG 421) will satisfy this endpoint.

Response: Study protocol information and results data have been included.

Ecological Effects (fish, invertebrates, and algae)

Acute toxicity to fish, invertebrates, and *algae*. The submitter provided aquatic toxicity data only for proposed analog chemicals that, as stated above, are not adequately similar to the sponsored chemical, or are incompletely identified (algal test). The ECOSAR values for the sponsored chemical are not appropriate because the ECOSAR model does not yet include a calculation for anionic dyes. Therefore, all three acute aquatic toxicity tests are needed on the sponsored chemical following OECD Test Guidelines.

The references provided for acute fish and invertebrate toxicity in the test plan text (Greim et al, 1994) do not match those in the robust summaries. In addition, the last structure in Table 3 of the test plan does not match the name provided, 2,2'-(1,2-ethenediyl)bis(5-aminobenzenesulfonic acid), dipotassium salt (the molecular structure shows nitro substituents while the name specifies amino groups).

Response: Two structurally related substituted azo colorants containing naphthalene sulfonic acid and benzene sulfonic acid residues and phenol contituents have been the subject of ecotoxicity studies in fish. Both exhibit a very low order of acute toxicity. The structural relative barium salt of 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methylbenzenesulfonic acid has been studied in two fish species (*Brachydanio rerio* and *Oryzias latipes*). The 96 hr- LC50 exceeded 500 mg/L, one in a semi-static test and the other in a static test (Hoechst AG, 1992). In other acute fish toxicity tests, the structurally related azo dye, 2-naphthalenecarboxylic acid, [(4-methyl-2-sulfophenyl)azo], calcium salt showed an 96-hr LC50=33 mg/L in Orange killifish (MITI, Japan, 1992). These data along with the database of information on more than 3000 dyes and pigments provides an adequate database to assess the ecotoxicity potential of Sunset Yellow.

Specific Comments on the Robust Summaries

Human Health Effects

Acute toxicity. Information missing from one or more of the robust summaries of the oral studies in rats and mice includes the purity of the test material, animal data (e.g., age and weight), dose levels tested, and method of LD_{50} calculation.

Repeated-dose toxicity. The robust summaries for the NTP 12-week (range-finding) dietary studies in rats and mice do not contain information on the specific hematology, clinical chemistry and urinalysis parameters that were examined, nor the specific organs that were weighed or examined for gross and microscopic pathology.

Genetic toxicity. *Gene mutations.* Information missing from a robust summary of an Ames test (Chung et. al., 1981) includes the purity of the test substance, test concentration levels (as opposed to a dose range),

culture conditions (e.g., temperature and medium used), duration of incubation, number of colonies counted per concentration, the source of the metabolic activation system, responses to positive controls, whether or not testing was conducted both with and without metabolic activation and the results of each of these test conditions, statistical methods used and the results of statistical analyses.

Chromosomal aberrations. Information missing from a robust summary of an *in vitro* chromosomal aberrations study (Ishidate *et al.*, 1984) includes test guideline/standardized method used, culture conditions (e.g., incubation temperature), actual test concentrations, and results of statistical analyses.

Response: These data have been added where available from the published or unpublished report.